Remarks

Based on the following remarks, Applicant respectfully requests that the Examiner reconsider the outstanding rejection and that it be withdrawn.

Rejection Under 35 U.S.C. § 103

In the Office Action at pages 4-11, the Examiner rejected claims 1, 4, 6, 8 and 11 under 35 U.S.C. § 103(a), as allegedly obvious over Seymour, A., *Manag. Care,* 10:11-16, 2001 (hereinafter "Seymour") in view of McCall, A.L., *Expert Opin. Pharmacother.* 2:699-713, 2001 (hereinafter "McCall"). Applicant respectfully traverses this rejection.

Obviousness determinations under 35 U.S.C. § 103 are carried out according to the standard set forth by the United States Supreme Court in *Graham v. John Deere Co. of Kansas City,* 383 U.S. 1, 148 U.S.P.Q. 459 (1966):

> [u]nder § 103, the scope and content of the prior art are to be determined; differences between the prior art and the claims at issue are to be ascertained; and the level of ordinary skill in the pertinent art resolved. Against this background, the obviousness or nonobviousness of the subject matter is determined. Such secondary considerations as commercial success, long felt but unsolved needs, failure of others, etc., might be utilized to give light to the circumstances surrounding the origin of the subject matter sought to be patented. As indicia of obviousness or nonobviousness, these inquiries may have relevancy.

Id. at 17-18, 148 U.S.P.Q. at 467.

In proceedings before the U.S. Patent and Trademark Office (USPTO), the Examiner bears the burden of establishing a *prima facie* case of obviousness based upon the prior art. *See In re Piasecki,* 223 U.S.P.Q. 785, 787-88 (Fed. Cir. 1984). Additionally, there must be a reason or rationale behind an obviousness determination

and "this analysis should be made explicit." *See KSR International Co. v. Teleflex, Inc.,*

127 S.Ct. 1727, 1741 (2007) (citing *In re Kahn,* 441 F.3d 977, 988, 78 U.S.P.Q.2d 1329,

1336 (Fed. Cir. 2006) ("[R]ejections on obviousness grounds cannot be sustained by

mere conclusory statements; instead, there must be some articulated reasoning with some

rational underpinning to support the legal conclusion of obviousness.")).

In response to *KSR*, the USPTO issued "Examination Guidelines for Determining

Obviousness Under 35 U.S.C. 103 in View of the Supreme Court Decision in *KSR*

International Co. v. Teleflex Inc." 72 Fed. Reg. 195, pp. 57526-35 (October 10, 2007)

(hereinafter "Guidelines"). The Guidelines reiterate and emphasize the Examiner's role

as a factfinder, using the factual inquiries set forth in *Graham*. Based on the fact record,

the Examiner must use "articulated reasoning with some rational underpinning to support

the legal conclusion of obviousness." Guidelines at page 57529 (internal citation

omitted).

> ***Seymour and McCall do not teach a composition comprising both glimepiride
> and metformin hydrochloride in the same composition, let alone a composition
> comprising a synergistic combination of glimepiride and metformin
> hydrochloride at the specified weight ratios.***

The present claims recite a solid pharmaceutical composition comprising a

synergistic combination of glimepiride and metformin hydrochloride, wherein the weight

ratio of glimepiride and metformin hydrochloride is about 1/500 (claim 1), or a method

of controlling blood glucose levels in a patient with type 2 diabetes, comprising

administering to said patient a synergistic combination of glimepiride and metformin

hydrochloride, in a solid dosage form, wherein the weight ratio of glimepiride and

metformin hydrochloride is about 1/500 (claim 8). Claims 4 and 6 depend from claim 1

and claim 11 depends from claim 8. These dependent claims are patentable for at least the reasons set forth for claims 1 and 8 below.

Seymour provides a clinical overview for the treatment of diabetes with glyburide and metformin hydrochloride as an initial therapy or as a replacement therapy for patients who are inadequately controlled on monotherapy. *See, e.g.*, Seymour at page 16. Seymour further provides recommended Glucovance® (glyburide and metformin hydrochloride) starting doses for initial and replacement therapy (Table 2) and a conversion guide for switching patients, for example, from Amaryl® (glimepiride) and Glucophage® (metformin hydrochloride) dual therapy to Glucovance® (Table 1). As such, Seymour does not teach a composition comprising both glimepiride and metformin hydrochloride in the same composition. In addition, Seymour does not teach the claimed synergistic combination of glimepiride and metformin hydrochloride, as noted by the Examiner. *See* Office Action at page 4.

The Examiner relies on the disclosure of McCall to cure these deficiencies, asserting that McCall teaches, for example, that: (1) glimepiride is approved for use with metformin; (2) glimepiride can be given at a dose of 1-8 mg as a monotherapy; and (3) glimepiride may have certain advantages over glyburide. *See* Office Action at page 5. However, McCall does not cure the deficiencies of Seymour because it does not teach a composition comprising both glimepiride and metformin hydrochloride in the same composition, let alone a composition comprising a synergistic combination of glimepiride and metformin hydrochloride at the specified weight ratios. Thus, for at least these reasons, Seymour and McCall cannot be used to support a *prima facie* case of obviousness of the claims.

Seymour teaches away from the use of glimepiride and metformin hydrochloride.

When references teach away from making a claimed combination, there can be no expectation of success in combining the references to support a *prima facie* case of obviousness. *See* Guidelines at page 57529. As described above, Seymour provides recommended Glucovance® (glyburide and metformin hydrochloride) starting doses for initial and replacement therapy (Table 2) and a conversion guide for switching patients, for example, from glimepiride and metformin hydrochloride dual therapy to Glucovance® (Table 1). As such, Seymour teaches switching patients to glyburide and metformin hydrochloride therapy, and therefore teaches away from glimepiride and metformin hydrochloride therapy. Accordingly, for at least these reasons, Seymour cannot be used to support a *prima facie* case of obviousness of the claims.

The Examiner has not provided the requisite rationale for why the claimed subject matter would have been obvious over Seymour in view of McCall.

In explaining the obviousness rejection, the Examiner asserts that it would have been obvious to substitute the glyburide component disclosed in Seymour with the glimepiride component disclosed in McCall, because it was known that glimepiride was associated with reduced hypoglycemic adverse effects. *See* Office Action at page 5. In addition, the Examiner asserts that one would have been motivated to combine the references because of the "individually known common utility" of the components of the references. Office Action at pages 5 and 6. Regardless of whether or not one would have been motivated to substitute glyburide with glimepiride, Applicant submits that one of ordinary skill in the art at the time the present application was filed would not have expected such a substitution would result in a synergistic combination of glimepiride and

metformin hydrochloride, because, as mentioned above, Seymour and McCall do not teach or suggest the claimed synergistic combination of glimepiride and metformin hydrochloride. In addition, art available at the time the present application was filed confirms that one of ordinary skill in the art would not have expected the claimed glimepiride and metformin hydrochloride composition would result in a synergistic combination. For example, De Fronzo, R.A., *Ann. Intern. Med.*, 131:281-303, 1999 (provided in the accompanying Information Disclosure Statement as document NPL8) discloses that clinical trials had not demonstrated the superiority of one sulfonylurea over another when given in maximally effective doses, and that clinical trials had shown glyburide and glimepiride exert equipotent glucose-lowering effects. *See, e.g.,* De Fronzo at page 289.

In explaining the obviousness rejection, the Examiner further asserts that it would have been obvious to a person of skill in the art at the time the invention was made to substitute the glyburide component of Glucovance® as taught by Seymour with glimepiride as taught by McCall because "it is routine in the medical arts to combine drugs that are known to have the same therapeutic utility and both metformin hydrochloride and glimepiride are known hypoglycemic drugs ... ." Office Action at pages 6 and 7. As such, the Examiner's rationale relies primarily on the assertion that a person of ordinary skill in the art would substitute glimepiride from all available sulfonylurea agents in a formulation containing metformin hydrochloride, and would then determine alternative dosages of metformin hydrochloride and glimepiride to arrive at dosages within the claimed weight ratios, because such dosages were individually known.

A statement that modifications of the prior art to meet the claimed invention would have been "well within the ordinary skill of the art at the time the claimed invention was made" because the references relied upon teach that all aspects of the claimed invention were individually known in the art is <u>not</u> sufficient to establish a *prima facie* case of obviousness without some objective reason to combine the teachings of the references. *See* M.P.E.P. § 2143.01; *Ex parte Levengood,* 28 U.S.P.Q.2d 1300 (Bd. Pat. App. & Inter. 1993). "[R]ejections on obviousness cannot be sustained by mere conclusory statements; instead, there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness." *KSR,* 82 U.S.P.Q.2d at 1396 quoting *In re Kahn,* 441 F.3d 977, 988 (Fed. Cir. 2006). Furthermore, it is never appropriate to rely solely on "common knowledge" in the art without evidentiary support in the record, as the principal evidence upon which a rejection is based. *See* M.P.E.P. § 2144.03(a); *In re Zurko,* 258 F.3d 1379, 1385 (Fed. Cir. 2001) ("[T]he Board cannot simply reach conclusions based on its own understanding or experience-or on its assessment of what would be basic knowledge or common sense. Rather, the Board *must point to some concrete evidence* in the record in support of these findings." (emphasis added)). As the court held in *Zurko,* an assessment of basic knowledge and common sense that is not based on any evidence in the record lacks substantial evidence support. *Id.* In addition, the U.S. Supreme Court has held that "rejections on obviousness cannot be sustained by *mere conclusory statements*; instead, there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness." *KSR,* 82 U.S.P.Q.2d at 1396 quoting *In re Kahn,* 441 F.3d 977, 988 (Fed. Cir. 2006).

Applicant respectfully submits that the Examiner has not provided any concrete evidence in the record that one of ordinary skill in the art would arrive at a synergistic combination of glimepiride and metformin hydrochloride at the specified weight ratio in view of the cited references. Rather, the Examiner has simply made an unsupported, conclusory statement that cannot and does not support a *prima facie* case of obviousness. *See KSR*, 127 S.Ct. at 1741. To combine references without evidentiary support constitutes impermissible hindsight. *See KSR*, 127 S. Ct. at 1740-41 ("A patent composed of several elements is not proved obvious merely by demonstrating that each element was, independently, known in the prior art."). Applicant respectfully submits that the Examiner has used impermissible hindsight to arrive at the conclusion that the claims are obvious over Seymour in view of McCall and has therefore not established a *prima facie* case of obviousness.

M.P.E.P. § 2141 provides exemplary rationales that may support a conclusion of obviousness. Of those, it appears that the Examiner may be alleging that the claimed subject matter was "obvious to try" because one of ordinary skill in the art purportedly could choose from a finite number of identified, predictable solutions in the art with a reasonable expectation of success. *See* M.P.E.P. § 2141(III)(E). Applicant respectfully disagrees that the claimed synergistic combination of glimepiride and metformin hydrochloride at the specified weight ratio would be predictable, as detailed below.

For at least these reasons, Applicant submits that the Examiner has not established a *prima facie* case of obviousness over the cited references, and therefore respectfully requests that this rejection be withdrawn.

Obviousness of the claimed subject matter cannot be predicated on an unknown or allegedly inherent property of the art.

Obviousness cannot be based on unknown properties of a composition. *See In re Ehrreich*, 590 F.2d 902, 909 (C.C.P.A. 1979) ("The question in a § 103 case is what the references would collectively suggest to one of ordinary skill in the art."); *see also In re Spormann*, 363 F.2d 444, 448 (C.C.P.A. 1966). "That which may be inherent is not necessarily known . . . [and] [o]bviousness cannot be predicated on what is unknown." *In re Spormann*, 363 F.2d at 448; *see also In re Adams*, 356 F.2d 998 (C.C.P.A. 1974). The law of anticipation by inherency, *i.e.*, lack of novelty, must be clearly distinguished from the law of obviousness. *Jones v. Hardy*, 727 F.2d 1524, 1529 (Fed. Cir. 1984). "[T]hough anticipation is the epitome of obviousness, [anticipation and obviousness] are separate and distinct concepts." *Id.*

At page 8 of the Office Action, the Examiner alleges that, because the art encompasses a fixed dose combination comprising glimepiride and metformin hydrochloride in a weight ratio of 1/500, and the claims encompass a fixed dose combination comprising glimepiride and metformin hydrochloride in a weight ratio of 1/500, one would reasonably expect that a combination comprising glimepiride and metformin hydrochloride in a weight ratio of 1/500 would exhibit "a synergistic combination." As explained above, Applicant disagrees that the art cited by the Examiner encompasses a fixed dose combination comprising both glimepiride and metformin. Moreover, the Examiner appears to allege that the synergistic combination of the claimed compositions is an inherent property of a composition comprising

glimepiride and metformin hydrochloride in a weight ratio of 1/500 that is allegedly known in the art.

Applicant respectfully reminds the Examiner that there is no such thing as inherent obviousness, since inherency and obviousness are different legal concepts. *See In re Spormann*, 363 F.2d 444 (C.C.P.A. 1966); *In re Rijckaert*, 9 F.3d 1531 (Fed. Cir. 1993). That which is inherent cannot be obvious, since inherent information "is not necessarily known . . . . [and] [o]bviousness cannot be predicated on what is unknown." *In re Spormann*, 363 F.2d at 448. For at least these reasons, Applicant respectfully asserts that the Examiner has not established a *prima facie* case of obviousness for the claims.

In sum, based on facts in the record, Applicant asserts that the Examiner has failed to establish a *prima facie* case of obviousness for at least the following reasons: (1) the Examiner has not established that the references teach all the elements of the claims, particularly a composition comprising glimepiride and metformin hydrochloride, or a composition comprising a synergistic combination of glimepiride and metformin at the specified weight ratios; (2) Seymour teaches away from the use of glimepiride and metformin hydrochloride, and therefore cannot be used to support a *prima facie* case of obviousness of the claims; (3) the Examiner has not provided the requisite rationale to combine the cited references to arrive at the claimed subject matter; and (4) obviousness of the claimed subject matter cannot be predicated on an unknown or allegedly inherent property of the art. Therefore, Applicant respectfully requests reconsideration and withdrawal of this rejection.

Even if prima facie obviousness were established, evidence of unexpected results exists which would overcome such a rejection.

Secondary considerations of non-obviousness include unexpected results. *Graham v. John Deere Co.*, 383 U.S. 1, 17, 86 S. Ct. 684, 694, 148 U.S.P.Q. 459, 467 (1966). The Federal Circuit has recently reaffirmed that the USPTO must in all cases consider any evidence presented by Appellants tending to support secondary considerations of non-obviousness. *In re John B. Sullivan and Findlay E. Russell*, 498 F.3d 1345, 84 U.S.P.Q.2d 1034 (Fed. Cir. 2007). As discussed above, the Examiner has not established a *prima facie* case of obviousness with respect to the claims. Moreover, the record demonstrates that *prima facie* obviousness, even if it were established, would be negated by the unexpected properties of the claimed invention, namely, the inventor's discovery of the claimed combination of glimepiride and metformin hydrochloride at a weight ratio of about 1/500.

Here, the Applicant has discovered that compositions comprising a combination of glimepiride and metformin hydrochloride at a weight ratio of about 1/500 have an unexpectedly improved effect on reducing blood glucose levels in diabetic patients. *See, e.g.*, paragraphs [0012], [0014] and [0033] of the substitute specification filed on July 26, 2004 (hereinafter "specification"). As disclosed in the specification, the treatment of patients with compositions comprising a combination of glimepiride and metformin at a weight ratio of 1/500 resulted in unexpected decreases in glycosylated hemoglobin (HbA$_{1c}$), fasting plasma glucose levels, and prandial blood glucose levels compared to treatments with glimepiride or metformin alone. *See, e.g.*, paragraph [0033] of the specification. These data are reproduced in the table below, along with data from U.S.

Patent No. 6,011,049 which measured the same parameters following treatment with

metformin and glyburide:

	Metformin 500 mg	Glimepiride 1 mg	Metformin + Glimepiride 500 mg / 1mg	Metformin + Glyburide 1500 mg / 20 mg (U.S. Pat. No. 6,011,049)
Glycosylated Hemoglobin (HbA$_{1c}$)	+0.06	+0.25	-0.70	+0.10
Fasting Plasma Glucose (FPG)	+0.75	+0.68	-1.77	+6.0
Prandial Blood Glucose	+1.08	+0.99	-2.7	*not determined*

Specifically, the above data show that treatment with compositions comprising

glimepiride and metformin, at a 1/500 weight ratio, resulted in a potentiated therapeutic

efficacy that is unexpectedly greater than treatment with glimepiride or metformin alone.

This potentiated effect was not observed with combined treatment of glyburide and

metformin at significantly increased doses (20 times more sulfonylurea and three times

more metformin). Thus, in view of the potentiated therapeutic efficacy observed with

compositions comprising glimepiride and metformin at a 1/500 weight ratio, the claimed

combinations of glimepiride and metformin would not have been predictable. As such,

even if a *prima facie* case of obviousness were established, which it has not, Applicant

respectfully contends that these unexpected results would be sufficient to overcome such

a rejection.

Exhibits A and B

As further evidence in support of the non-obviousness of the claimed subject

matter, Applicant reminds the Examiner of Exhibit A (González-Oritz *et al.*, *Rev. Inv.*

Clin. 56:327-333, 2004; *see* paragraph [0012] of the specification) submitted with the

Amendment and Reply Under 37 C.F.R. § 1.116 filed on April 6, 2009. Exhibit A is also

provided in the accompanying Information Disclosure Statement with an English

language translation as documents NPL11 and NPL12. As explained in the Abstract,

Exhibit A provides data from a clinical study involving the treatment of diabetic patients

with 4 mg of glimepiride alone, 2 g of metformin alone, or 4 mg of glimepiride and 2 g

of metformin (1/500 weight ratio) together in the same composition. The efficacy

criteria evaluated in the study were either a decrease in glycosylated hemoglobin

(HbA$_{1C}$, or "A1C" in Exhibit A) of 1% or more, or a reduction in A1C of 7% or less.

According to the Abstract, the decrease in A1C concentration was -0.9 ± 1.6% in the

glimepiride group, -0.7 ± 2.1% in the metformin group, and -1.3 ± 1.8 mg/dL in the

combined therapy group. Thus, only the combination therapy group attained the efficacy

criteria of decreased A1C of 1% of more. Furthermore, the percentage of patients

attaining the efficacy criteria of 1% or more or a reduction in A1C of 7% or less was

markedly enhanced in the combination therapy group compared to the monotherapy

groups, while the frequency of adverse events was similar for all treatment groups.

Applicant also reminds the Examiner of Exhibit B (González-Oritz *et al.*, *J.*

Diabetes Complications, electronically published on October 10, 2008 ahead of print;

uncorrected proof) submitted with the Amendment and Reply Under 37 C.F.R. § 1.116

filed on April 6, 2009 as additional evidence in support of non-obviousness. Exhibit B is

also provided in the accompanying Information Disclosure Statement as document

NPL13. Exhibit B provides data from a clinical study comparing the efficacy of

glycemic control in patients treated with 1 mg of glimepiride and 500 mg of metformin

(1/500 weight ratio) together in the same composition, to patients treated with 5 mg of

glibenclamide (glyburide) and 500 mg of metformin together in the same composition. As detailed in Table 2, the percentage of patients who maintained glycemic control after 12 months of treatment, measured by A1C levels less than 7%, were markedly higher in the glimepiride/metformin treatment groups compared to the glyburide/metformin treatment groups.

In sum, Exhibits A and B provide further support for the non-obviousness of the claimed subject matter because they provide evidence that the claimed combination of glimepiride and metformin unexpectedly met certain therapeutic efficacy criteria compared to monotherapy with glimepiride or metformin alone, or compared to combination therapy with metformin and another sulfonylurea agent. Therefore, even if a *prima facie* case of obviousness were established, which it has not, Applicant respectfully contends that these unexpected results would be sufficient to overcome such a rejection. For at least these reasons, Applicant respectfully requests this rejection be withdrawn.

Exhibit C

As further evidence in support of the non-obviousness of the claimed subject matter, Applicant submits Exhibit C. Exhibit C provides post-filing date data from a clinical study involving the treatment of patients with type II diabetes with a daily dose of 4 mg of glimepiride alone ("Glimepiride"), 2 g of metformin hydrochloride alone ("Metformin"), or a combination of 4 mg of glimepiride and 2 g of metformin hydrochloride (1/500 weight ratio) together in the same composition ("Glimepiride + Metformin"). Glycosylated hemoglobin (HbA_{1C}) levels were measured prior to treatment ("baseline") and the percent change in HbA_{1C} levels was determined by

comparing baseline HbA$_{1C}$ levels to HbA$_{1C}$ levels after treatment. The combination therapy group attained a reduction of HbA$_{1C}$ levels greater than the individual effects of glimepiride or metformin hydrochloride treatment alone. Moreover, only the combination therapy group attained the efficacy criteria of decreased HbA$_{1C}$ of 1% of more.

As such, Exhibit C provides further support for the non-obviousness of the claimed subject matter because it provides evidence of the synergistic effects of the claimed combination of glimepiride and metformin hydrochloride compared to monotherapy with glimepiride or metformin hydrochloride alone. Therefore, even if a *prima facie* case of obviousness were established, which it has not, Applicant respectfully contends that these unexpected results would be sufficient to overcome such a rejection. For at least these reasons, Applicant respectfully requests this rejection be withdrawn.

Exhibit D

As further evidence in support of the non-obviousness of the claimed subject matter, Applicant submits Exhibit D (Shimpi, R.D., *et al.*, *Int. J. PharmTech Res.* 1: 50-61, 2009). Exhibit D is also provided in the accompanying Information Disclosure Statement as document NPL27. Exhibit D provides data from a clinical study comparing the effects of metformin and glimepiride treatment with metformin and glibenclamide (glyburide) treatment in patients with type 2 diabetes. Specifically, patients were treated with compositions comprising 1000 mg of metformin and 10 mg of glyburide, or with compositions comprising 1000 mg of metformin and 2 mg of glimepiride (1/500 weight ratio). *See, e.g.,* Exhibit D at page 53. After 12 weeks of treatment, reductions in HbA$_{1C}$

levels were observed in both treatment groups; however, patients treated with metformin

and glimepiride exhibited significantly reduced HbA$_{1C}$ levels compared to patients

treated with metformin and glyburide. *See, e.g.,* Exhibit D at Figure 1. In addition,

patients treated with metformin and glimepiride exhibited significantly reduced fasting

plasma glucose (FPG) levels compared to patients treated with metformin and glyburide.

See, e.g., Exhibit D at Figure 2.

As such, Exhibit D provides further support for the non-obviousness of the

claimed subject matter because it provides evidence that the claimed compositions

comprising glimepiride and metformin at the specified weight ratios unexpectedly met

certain therapeutic efficacy criteria compared to therapy with metformin and glyburide.

Therefore, even if a *prima facie* case of obviousness were established, which it has not,

Applicant respectfully contends that these unexpected results would be sufficient to

overcome such a rejection. For at least these reasons, Applicant respectfully requests

this rejection be withdrawn.

Conclusion

The stated ground of rejection has been properly traversed. Applicant therefore respectfully requests that the Examiner reconsider the presently outstanding rejection and that it be withdrawn. Applicant believes that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Reply is respectfully requested.

Respectfully submitted,

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Exhibit A

RIC

Eficacia y seguridad de la terapia hipoglucemiante oral combinada de glimepirida más metformina en una sola forma farmacéutica en pacientes con diabetes mellitus tipo 2 y falla secundaria a monoterapia con glibenclamida

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Efficacy and safety of glimepiride plus metformin in a single presentation, as combined therapy, in patients with type 2 diabetes mellitus and secondary failure to glibenclamide, as monotherapy

ABSTRACT

Objective. To evaluate the efficacy and safety of glimepiride plus metformin in a single presentation, as combined therapy, in patients with type 2 diabetes mellitus (DM2) with secondary failure to glibenclamide. Material and methods. A randomized, double-blind, multicentric trial was carried out in 104 obese patients with DM2, fasting glucose >140 mg/dL and glycated hemoglobin A1c (A1C) > 8%, in spite of treatment with glibenclamide at maximum doses and medical nutrition therapy for at least the 3 months previous to the study. After randomization, the patients received in titrated way during 3 months one of the following treatments: up to 4 mg of glimepiride, 2 g of metformin or 4 mg of glimepiride plus 2 g of metformin in a single presentation. Efficacy criteria were either a decrease in A1C of 1% or more, or a reduction in A1C of 7% or less. Adverse events were carefully monitored during the study. Results. At the end of the study, the decrease in A1C concentration was -0.9 ± 1.6% (CI 95%: -0.2 to -1.5) in the glimepiride group, -0.7 ± 2.1% (CI 95%: 0.2 to -1.6) in the metformin group, and -1.3 ± 1.8 mg/dL (CI 95%: -0.6 to -1.9) in the combined therapy group. The percentage of patients that showed a decrese in A1C of 1% or higher was 35.1, 21.2 and 47.0% in the glimepiride, in the metformin and in the combined therapy groups, respectively (p < 0.001). The percentage of patients with decreased A1C of 7% or less was 18.9, 9.0 and 23.5% in the glimepiride, in the metformin and in the combined therapy groups, respectively (p = 0.01). The frequency of adverse events was similar for all the

RESUMEN

Objetivo. Evaluar la eficacia y la seguridad de la terapia oral combinada de glimepirida más metformina en una sola forma farmacéutica en pacientes con diabetes mellitus tipo 2 (DM2) y falla secundaria a monoterapia con glibenclamida. **Material y métodos.** Se llevó a cabo un ensayo clínico multicéntrico, doble ciego, al azar, en 104 pacientes obesos, con DM2, que habían recibido monoterapia con glibenclamida a dosis máximas y terapia médica de nutrición durante al menos tres meses, y que presentaban glucosa de ayuno >140 mg/dL y hemoglobina glucosilada A1c (A1C) > 8%. Los pacientes recibieron durante tres meses en forma titulada hasta 4 mg de glimepirida, 2 g de metformina o 4 mg de glimepirida más 2 g de metformina en una sola presentación. Se consideraron como criterios de eficacia la disminución de 1% o más de A1C o su reducción a 7% o menos. Los efectos indeseables se vigilaron en cada visita. **Resultados.** Al final del estudio la disminución en la A1C en el grupo de glimepirida fue de -0.9 ± 1.6 (IC 95%: -0.2 a -1.5), en el de metformina de -0.7 ± 2.1% (IC 95%: 0.2 a -1.6) y en el de la combinación de -1.3 ± 1.8% (IC: -0.6 a -1.9). El porcentaje de pacientes que logró disminuir la A1C en 1% o más después del tratamiento fue 35.1, 21.2 y 47.0% para los grupos de glimepirida, metformina y combinación, respectivamente (p < 0.001). El porcentaje de pacientes que disminuyó la A1C a 7% o menos al final de la investigación fue 18.9, 9.0 y 23.5% para los grupos de glimepirida, metformina y combinación, respectivamente (p = 0.01). La frecuencia de efectos adversos fue similar en los tres grupos. **Conclusión.** La combinación de glimepirida más metformina en una sola forma farmacéutica durante tres meses fue eficaz y segura en pacientes con DM 2 y falla secundaria a monoterapia con glibenclamida.

*groups. **Conclusion.** The combined use of glimepiride plus metformin in a single presentation for 3 months showed to be efficacious and safe in patients with DM2 and secondary failure to glibenclamide.*

Key words. *Glimepiride. Metformin. Diabetes mellitus type 2 treatment. Oral antidiabetic drugs combined therapy.*

Palabras clave. Glimepirida. Metformina. Tratamiento para diabetes mellitus tipo 2. Combinaciones de antidiabéticos orales.

INTRODUCCIÓN

La enfermedad arterial coronaria es en la actualidad una de las principales causas de mortalidad en el adulto en la mayoría de los países industrializados, así como en algunos países de América Latina y en México.[1] El incremento de la cardiopatía isquémica en México se debe en gran parte a una elevada prevalencia de obesidad, diabetes mellitus tipo 2 (DM2), hipertensión arterial y dislipidemia; enfermedades que comparten como un mecanismo patogénico común al proceso de aterogénesis.[2] La DM2, en forma particular, incrementa la mortalidad general debido a que la mayoría de las estrategias encaminadas a mejorar su control fracasan por el inadecuado diagnóstico de la enfermedad y por la falta de adherencia a los regímenes terapéuticos indicados, lo que trae por consecuencia la aparición de complicaciones, disminución en la calidad de vida del paciente y un mayor gasto en los servicios de salud.[3]

Se ha establecido que muchas de las complicaciones crónicas de la DM2 tienen su origen en disturbios bioquímicos y celulares que anteceden a la aparición de las lesiones patológicas características en los tejidos afectados o de la enfermedad clínica manifiesta.[4,5] Evidencias experimentales vinculan a la hiperglucemia, que es el hallazgo patognomónico y cardinal de la diabetes, con las complicaciones. Dichas evidencias han mostrado que los regímenes intensivos para el control de la glucosa pueden disminuir el riesgo de aparición de las complicaciones, sin embargo, se ha observado que la implementación y el mantenimiento de tales regímenes son difíciles de llevar a cabo por la gran mayoría de los pacientes diabéticos.[6]

En los últimos años, numerosos agentes hipoglucemiantes orales se han desarrollado para tratar de incidir en los diferentes mecanismos y vías que conllevan a la DM2 y en la actualidad contamos con numerosas alternativas de tratamiento. De acuerdo con las recomendaciones generales para el control de la DM2, la terapia hipoglucemiante oral combinada es la elección a seguir en caso de una respuesta inadecuada a monoterapia.[7] Se menciona que un porcentaje variable de pacientes con diabetes requerirán de un segundo hipoglucemiante oral en algún momento dado de su vida. Existe evidencia que demuestra que la suma de un segundo agente antidiabético es efectiva para mejorar el control metabólico, sin embargo, no queda claro cuál sería el mejor fármaco a combinar.[8]

La falla secundaria observada en el grupo específico de las sulfonilureas se calcula que varía de 5 a 10% por año de uso, independientemente de la adherencia al tratamiento, la ganancia de peso y las enfermedades intercurrentes de los pacientes.[9] La literatura médica muestra que la terapia de combinación de alguna sulfonilurea más metformina ha logrado, en poblaciones de diferentes características, disminuir la concentración de hemoglobina glucosilada A1c (A1C) hasta en 2%.[10,11] Por otra parte, la combinación de una sulfonilurea más metformina va orientada a actuar sobre dos de los principales mecanismos fisiopatológicos de la diabetes mellitus tipo 2, que son la falla en la secreción de insulina y la resistencia periférica a dicha hormona,[12] por lo que la asociación sinérgica parece ser una buena alternativa terapéutica, principalmente para aquellos pacientes con hiperglucemia persistente.

El objetivo de la presente investigación fue evaluar la eficacia y la seguridad de la terapia hipoglucemiante oral combinada de glimepirida más metformina en una sola forma farmacéutica en pacientes con DM2 y falla secundaria a monoterapia con glibenclamida.

MATERIAL Y MÉTODOS

Se llevó a cabo un ensayo clínico multicéntrico, doble ciego, al azar, en 104 pacientes adultos (40 a 65 años) con DM2. Todos habían recibido monoterapia con glibenclamida a dosis máximas (20 mg/día) y terapia médica de nutrición de acuerdo a los criterios recomendados por la Asociación Americana de Diabetes[13] durante al menos tres meses y que presentaban glucosa de ayuno >140 mg/dL y A1C > 8%, por lo que se les consideró con falla secundaria a glibenclamida. Los pacientes firmaron la hoja de consentimiento informado, previa lectura y explicación por parte del investigador. El estudio reunió los

requisitos necesarios para realizar investigación en seres humanos y el proyecto fue previamente autorizado por los Comités de Investigación y Ética del Hospital de Especialidades del Centro Médico Nacional de Occidente del Instituto Mexicano del Seguro Social. Se incluyeron pacientes con índice de masa corporal (IMC) > 27 kg/m², con capacidad para la deglución y que hubieran dado su consentimiento voluntario por escrito. No se incluyeron pacientes con hiperglucemia grave (> 270 mg/dL), sospecha de embarazo, tratamiento con insulina, intolerancia o alergia conocidas a sulfonilureas o biguanidas, o con presencia de insuficiencia cardiaca, renal, cardiopatía isquémica, enfermedad vascular cerebral, neuropatía visceral, hepatopatía crónica o infección por el virus de la inmunodeficiencia humana. No se permitió la ingesta de medicamentos que presentaran interacción farmacológica con glimepirida o metformina, tales como acetazolamida, ácido nicotínico, ácido para amino salicílico, analgésicos antiinflamatorios no esteroideos, antagonistas 2 de histamina, barbitúricos, ciclofosfamida, clonidina, cloramfenicol, cumarínicos, disopiramida, epinefrina, estatinas, fenfluramina, fenotiacina, fibratos, fluoxetina, guanetidina, hormonas esteroides, ifosfamida, inhibidores de la monoaminooxidasa, laxantes, miconazol, quinolonas, reserpina, rifampicina, sulfamidas y tetraciclinas. Se consideraron como criterios de exclusión del estudio: presencia de hipoglucemia grave (< 60 mg/dL y la necesidad de ayuda de un tercero para su recuperación) aun con el empleo de las dosis mínimas, efectos indeseables intolerables, falta de adhesión al tratamiento médico (ingesta del medicamento < 80%), inasistencia a las visitas programadas (falta a la cita en >1 ocasión), enfermedad intercurrente o accidente que ameritara hospitalización, ingesta durante el estudio de medicamentos con interacción farmacológica con glimepirida o metformina y decisión de retiro voluntario. Se consideraron como criterios de eficacia la disminución de 1% o más de A1C o su reducción a 7% o menos.

Se realizó un periodo de lavado de una semana, donde a todos los pacientes se les suspendió la glibenclamida y se les hizo hincapié en seguir la terapia médica de nutrición a lo largo del estudio, donde las calorías se calcularon de acuerdo con su peso corporal con el propósito de llevar o mantener al paciente en el peso ideal. La adherencia a la terapia médica de nutrición se evaluó en cada cita programada a lo largo del estudio por medio de un recordatorio de alimentos de 24 horas. Se dio la indicación de continuar con la actividad física acostumbrada. Posterior al periodo de lavado, los pacientes fueron asignados al azar a alguno de los siguientes grupos de intervención farmacológica: glimepirida, metformina o la combinación de glimepirida más metformina. De acuerdo con la concentración de la glucemia basal la dosis fue prescrita de forma titulada como se señala a continuación: hasta 199 mg/dL los pacientes recibieron un comprimido de 2 mg de glimepirida o un comprimido de 1 g de metformina o un comprimido de 2 mg de glimepirida más 1 g de metformina en la misma presentación cada 24 horas; de 200 hasta 270 mg/dL recibieron 4 mg de glimepirida en dos comprimidos o 2 g de metformina en dos comprimidos o 4 mg de glimepirida más 2 g de metformina en la misma presentación en dos comprimidos, divididos en dos tomas al día. Los frascos contenedores de los medicamentos fueron idénticos y preparados por el laboratorio farmacéutico patrocinador con asignación de códigos, los cuales se conocieron hasta el final de la investigación. Durante el estudio, una persona sin conflicto de interés realizó el conteo de medicamento para la evaluación de apego al tratamiento.

Al inicio y 90 días después de la intervención farmacológica se investigaron el peso, la talla, se calculó el IMC con la fórmula: peso (kg)/[talla (m)]² y se determinaron las siguientes mediciones de laboratorio: glucemia de ayuno, A1C, insulina, colesterol total, colesterol de las lipoproteínas de alta densidad (C-HDL), triglicéridos, creatinina, ácido úrico, transaminasa glutámico oxalacética (TGO), transaminasa glutámico pirúvica (TGP) y deshidrogenasa láctica (DHL). A los 30 y 60 días del inicio del estudio se midieron la glucemia de ayuno, la A1C y la DHL.

Los efectos indeseables se registraron en cada visita en una hoja especial en donde se especificaron cada una de las manifestaciones clínicas que se consideraron como probables, posibles o directamente relacionadas con el uso de los fármacos ingeridos.

Se utilizaron técnicas enzimáticas (Ortho-clinical Diagnostics, Inc. Rochester, NY, USA) para las determinaciones de glucemia, colesterol total, C-HDL, triglicéridos, creatinina, ácido úrico, TGO, TGP y DHL, con coeficientes de variación intra e inter-ensayo menores al 3%. La A1C y la insulina fueron medidas por método enzimático de polarización fluorescente (Abbott Lab. Abbott Park. Il, USA), con coeficientes de variación intra e inter-ensayo menores de 5%.

ANÁLISIS ESTADÍSTICO

Para el cálculo del tamaño de la muestra se utilizó la fórmula correspondiente para ensayos clínicos,[14]

en la que se tomó en cuenta una confiabilidad de 95%, un poder estadístico del 80%, una desviación estándar de la hemoglobina A1C de 1.5% en población con diabetes mellitus tipo 2 y una diferencia esperada de 1.1% en la concentración de A1C, lo que dio por resultado 29 pacientes por grupo. Los resultados se reportan en promedio ± desviación estándar. Para el análisis de las variables cuantitativas se empleó la prueba de ANOVA y la t de Student. Para las variables cualitativas se utilizó la χ^2. Se calculó el intervalo de confianza al 95% (IC 95%). Se consideró un nivel de significación con una p < 0.05.

RESULTADOS

De los 104 pacientes, 51 fueron mujeres y 53 hombres. Las características clínicas y el perfil inicial de laboratorio fueron similares para los tres grupos de estudio (Cuadro 1). Después de los tres meses de la intervención, existió un incremento significativo del C-HDL después de la administración de glimepirida (Cuadro 1).

En la figura 1 se muestra la evolución de la glucosa a lo largo del estudio en los tres grupos de tratamiento, donde se encontró una tendencia a la dis-

Cuadro 1. Perfil clínico y de laboratorio inicial y 3 meses después de la intervención farmacológica.

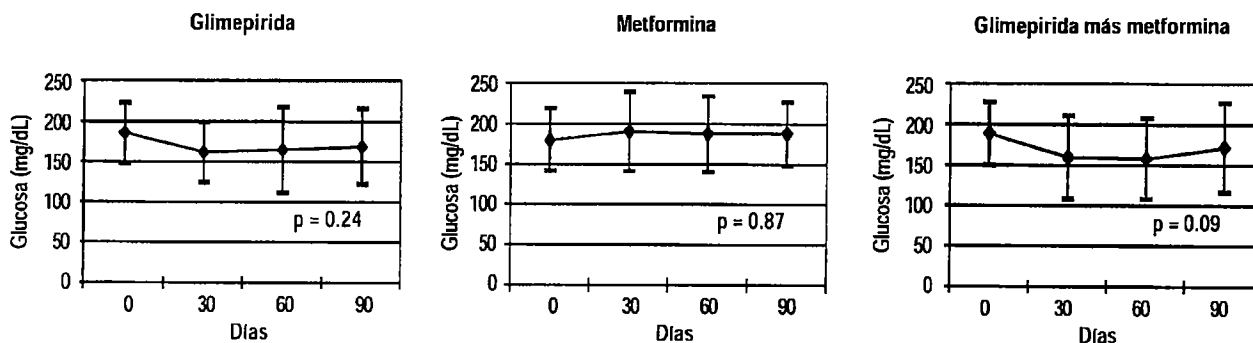|	Glimepirida n = 37		Metformina n = 33		Combinación n = 34	
	Inicial	3 meses	Inicial	3 meses	Inicial	3 meses
Edad, años	53 ± 8		53 ± 7		53 ± 7	
Evolución, años	7 ± 4		7 ± 5		6 ± 4	
IMC, kg/m²	29.9 ± 3.5	31.4 ± 8.5	31.3 ± 5.4	30.1 ± 4.2	31.3 ± 4.7	31.8 ± 5.0
Sistólica***	132 ± 17	126 ± 17	129 ± 15	119 ± 31	128 ± 9	128 ± 11
Diastólica***	79 ± 7	78 ± 9	80 ± 11	78 ± 8	81 ± 6	82 ± 6
Creatinina*	0.9 ± 0.3	0.8 ± 0.2	0.6 ± 0.1	0.7 ± 0.1	0.7 ± 0.2	0.8 ± 0.3
Ácido úrico*	4.2 ± 0.8	4.5 ± 1.3	4.3 ± 1.2	4.3 ± 1.1	4.2 ± 0.8	4.3 ± 0.7
Colesterol*	191 ± 49	199 ± 46	219 ± 40	208 ± 32	206 ± 41	201 ± 37
C-HDL*	36 ± 10	43 ± 13****	40 ± 9	44 ± 24	43 ± 12	39 ± 11
Triglicéridos*	195 ± 117	184 ± 119	280 ± 231	208 ± 101	205 ± 102	232 ± 168
Insulina**	14.1 ± 8.3	12.1 ± 7.3	9.3 ± 2.9	9.1 ± 4.7	13.4 ± 7.0	13.1 ± 12.2
TGO, U/L	29 ± 18	34 ± 21	41 ± 16	37 ± 24	26 ± 15	33 ± 25
TGP, U/L	34 ± 26	34 ± 22	49 ± 26	42 ± 20	33 ± 22	31 ± 21
DHL, U/L	310 ± 167	336 ± 161	404 ± 181	352 ± 125	322 ± 156	323 ± 142

IMC: índice de masa corporal, C-HDL: colesterol de las lipoproteínas de alta densidad, TGO: transaminasa glutámico-oxalacética. TGO: transaminasa glutámico-pirúvica, DHL: deshidrogenasa láctica.
* mg/dL.
** μU/mL.
*** mm Hg.
**** p = 0.01 entre inicial y 3 meses en el grupo de glimepirida.

Glimepirida   **Metformina**   **Glimepirida más metformina**

p = 0.24   p = 0.87   p = 0.09

Figura 1. Concentraciones de glucosa a lo largo de los tres meses de tratamiento en los grupos de glimepirida (n = 37), metformina (n = 33) y combinación (n = 34). Prueba estadística: ANOVA.

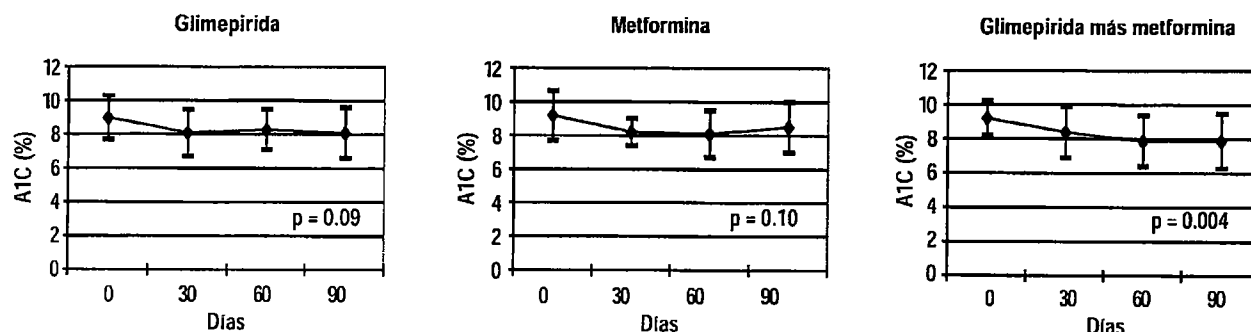**Figura 2.** *Concentraciones de hemoglobina A1C a lo largo de los tres meses de tratamiento en los grupos de glimepirida (n = 37), metformina (n = 33) y combinación (n = 34). Prueba estadística: ANOVA.*

minución en los pacientes que recibieron la combinación de glimepirida más metformina. La disminución de la glucosa en el grupo de glimepirida después de los tres meses de tratamiento fue de -16.7 ± 57.5 mg/dL (IC 95%: 6.3 a -39.7), en el grupo que recibió metformina de 8 ± 48.6 mg/dL (IC 95%: 29.8 a -13.8) y en el de la combinación de -16.8 ± 58.8 mg/dL (IC 95%: 5.3 a -38.9).

Tal como lo muestra la figura 2, a lo largo del estudio existió una disminución significativa en la A1C en el grupo que recibió la combinación de glimepirida más metformina y una tendencia a su reducción en el grupo de glimepirida. La disminución de la A1C en el grupo de glimepirida después de los tres meses de tratamiento fue de -0.9 ± 1.6% (IC 95%: -0.2 a -1.5), en el grupo que recibió metformina de -0.7 ± 2.1% (IC 95%: 0.2 a -1.6) y en el de la combinación de -1.3 ± 1.8 mg/dL (IC 95%: -0.6 a -1.9).

El porcentaje de pacientes que logró disminuir la A1C en 1% o más después de tres meses de tratamiento fue 35.1 (n = 13), 21.2 (n = 7) y 47.0% (n = 16) para los grupos de glimepirida, metformina y combinación, respectivamente, con una diferencia estadísticamente significativa (p < 0.001) entre los grupos; que persistió entre glimepirida y metformina (p = 0.02) y entre la combinación y metformina (p = 0.001), pero no entre glimepirida y la combinación (p = 0.08).

El porcentaje de pacientes que logró disminuir la A1C a 7% o menos después de tres meses de tratamiento fue 18.9 (n = 7), 9.0 (n = 3) y 23.5% (n = 8) para los grupos de glimepirida, metformina y combinación, respectivamente, con una diferencia estadísticamente significativa (p = 0.01) entre los grupos; que persistió entre glimepirida y metformina (p = 0.04) y entre la combinación y metformina (p = 0.004), pero no entre glimepirida y la combinación (p = 0.38).

Los efectos indeseables consistieron principalmente en molestias gastrointestinales y fueron similares (p = 0.50) en los tres grupos [24.3% (n = 9) para glimepirida, 36.3% (n = 12) para metformina y 26.4% (n = 9) para la combinación]. La hipoglucemia se presentó sólo en 2.7% (n = 1) en el grupo de glimepirida, en el 3.0% (n = 1) en el grupo con metformina y en el 2.9% (n = 1) en el de la combinación y fue considerada como leve en todos los casos. En ningún paciente se requirió la suspensión del tratamiento por efectos indeseables o por alguno de los criterios de exclusión contemplados previamente en la metodología. No existió diferencia estadísticamente significativa entre los tres grupos (p = 0.15) en el porcentaje de pacientes que presentaron al final del estudio hiperglucemia grave (glucosa > 270 mg/dL) y que requirieron de alguna otra opción terapéutica para el control de su diabetes [35.2% (n = 13) en el grupo de glimepirida, 42.3% (n = 14) en el de metformina y 20.6% (n = 7) en el de la combinación de metformina más glimepirida].

DISCUSIÓN

Los resultados del Estudio Prospectivo de Diabetes en el Reino Unido (UKPDS) nos muestran que más de la mitad de los pacientes que tienen más de seis años de evolución de la diabetes requerirán en términos generales de la adición de un segundo agente hipoglucemiante oral para lograr el control de la glucosa.[9] En el presente estudio, los pacientes incluidos se encontraban en esa parte de la historia natural de la enfermedad, donde la monoterapia con glibenclamida no era suficiente para lograr las metas de control y, por ende, se requería de la adición de un segundo medicamento.

Son pocos los estudios que comparan directamente la efectividad de la combinación de agentes anti-

diabéticos, sin embargo, los resultados de diversas investigaciones en la literatura médica, con una de las combinaciones más utilizadas por su efectividad y seguridad, como es la de sulfonilureas con metformina, nos muestran que con esta estrategia se ha logrado disminuir la A1C hasta 2% en relación con la concentración previa al tratamiento.[8,10,11]

Para nuestro estudio elegimos como representante de las sulfonilureas a una de tercera generación, la glimepirida, que además de incrementar la secreción de insulina, presenta efectos extrapancreáticos relacionados con un incremento en las moléculas que facilitan el transporte activo de glucosa en el músculo y en los adipocitos, con el resultante incremento del ingreso de glucosa a la célula.[15,16] La metformina, medicamento considerado a combinar con la glimepirida, se reconoce como un antihiperglucemiante oral perteneciente a la familia de las biguanidas que potencializa los efectos metabólicos de la insulina en los tejidos periféricos e inhibe la gluconeogénesis hepática, además de reducir las concentraciones de triglicéridos y colesterol y propiciar la pérdida de peso.[17,18]

La experiencia clínica previa con el empleo de la combinación de glimepirida más metformina sólo ha sido publicada por Charpentier[19] en un grupo de pacientes con falla secundaria a la monoterapia con metformina. Cabe mencionar que en la evaluación de la efectividad de una segunda droga es importante tomar en cuenta la concentración basal de glucosa, ya que no se tiene una clara evidencia sobre cuál es el punto de corte de la glucemia en el cual se deba de indicar un segundo medicamento. En nuestro estudio, al igual que en el mencionado anteriormente, la glucosa basal de los pacientes antes de recibir la combinación fue similar, lo que facilita la comparación de algunos de sus resultados. En la investigación francesa, la disminución en la A1C fue de 0.7%, que es menor a la encontrada en nuestros pacientes después de recibir la combinación de glimepirida más metformina o la glimepirida sola y similar a la obtenida con la metformina sola. Esto nos permite corroborar la eficacia de la combinación en el manejo del paciente de difícil control, ya que logró disminuir la A1C al menos en 1% en cerca de la mitad de los pacientes, sin embargo, debemos reconocer que sólo un pequeño a moderado porcentaje de pacientes logró la reducción de la A1C por debajo de 7%. Desafortunadamente, en la presente investigación sólo se evaluó la glucemia de ayuno, en la cual sólo existió tendencia a la reducción en el grupo de la combinación y no fue evaluada la glucemia posprandial, que constituye uno de los puntos que recomienda la Asociación Americana de Diabetes para estimar el logro de las metas del trata-

miento en el paciente diabético y cuya elevación, al igual que la hiperglucemia de ayuno, se asocian con un incremento en la A1C, sin embargo, con la reducción que se obtuvo en la muestra mexicana de A1C podemos presuponer una mejoría de la glucemia posprandial mayor que la publicada en la población francesa.[19] Cabe señalar, que otra limitante del estudio fue el corto periodo de manejo exclusivamente con dieta (una semana), aunque a los pacientes se les había prescrito terapia médica de nutrición al menos tres meses previos al estudio. Por otra parte, para la prescripción de las combinaciones se debe de tomar en cuenta las ventajas, desventajas y limitaciones de los medicamentos. En nuestra investigación, aproximadamente una tercera parte de los pacientes en los tres grupos requirió al final del estudio de la administración de insulina, al igual que en el grupo de Charpentier,[19] con lo que ratificamos el hecho de que no existe aún el medicamento ideal y de que la mayoría de los casos requerirán de una alternativa terapéutica adicional para alcanzar las metas de tratamiento.

Con excepción del C-HDL que aumentó en el grupo de glimepirida después del periodo de estudio, no se encontraron otros efectos metabólicos benéficos que se conocen de los dos fármacos utilizados, quizá debido al corto tiempo de la investigación o a las dosis empleadas.

Dentro de los efectos indeseables de la glimepirida se encuentran la hipoglucemia, los trastornos gastrointestinales y las reacciones alérgicas;[20] los reportados para la metformina son principalmente los gastrointestinales.[21] En nuestro estudio el porcentaje de los efectos adversos fue estadísticamente similar entre los tres brazos del estudio, lo que demuestra que esta combinación no presenta adición de los efectos indeseables de cada uno de sus componentes por separado. Por otra parte, debemos de señalar que ningún paciente requirió la suspensión del tratamiento debido a hipoglucemia y que existió buena adherencia al tratamiento.

En conclusión, la prescripción titulada de la combinación de glimepirida más metformina en una sola forma farmacéutica fue eficaz y segura en pacientes con DM2 y falla secundaria a monoterapia con glibenclamida, al disminuir la A1C después de tres meses de tratamiento y al ser bien tolerada durante el periodo de estudio.

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Exhibit B

Efficacy of glimepiride/metformin combination versus glibenclamide/metformin in patients with uncontrolled type 2 diabetes mellitus[☆]

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Abstract

Aim: The aim of this study was to compare the efficacy of glimepiride/metformin combination versus glibenclamide/metformin for reaching glycemic control in patients with uncontrolled type 2 diabetes mellitus. **Patients and Methods:** A randomized, double-blind, multicenter clinical trial was performed in 152 uncontrolled type 2 diabetic patients. Serum fasting and postprandial glucose, hemoglobin A1c (A1C), high-density lipoprotein cholesterol, and triglycerides were measured. After random allocation, all patients received two pills of glimepiride (1 mg)/metformin (500 mg) or glibenclamide (5 mg)/metformin (500 mg) po once a day. Dosage was increased to a maximum of four pills in order to reach the glycemic control goals (fasting glucose ≤7.2 mmol/l, postprandial glucose <10.0 mmol/l, A1C <7%, or an A1C ≥1% reduction). Statistical analyses were carried out using chi-square, ANOVA, or Student's t test. The protocol was approved by an ethics committee and met all requirements needed to perform research in human subjects; all patients gave written informed consent. **Results:** Each study group included 76 patients. No significant differences in basal clinical and laboratory characteristics between groups were found. At the end of the study, A1C concentration was significantly lower in the glimepiride/metformin group ($P=.025$). A higher proportion of patients from the glimepiride group (44.6% vs. 26.8%, $P<.05$) reached the goal of A1C <7% at 12 months of treatment. A higher proportion of hypoglycemic events were observed in the glibenclamide group (28.9% vs. 17.1%, $P<.047$). **Conclusion:** Glimepiride/metformin demonstrated being more efficacious than glibenclamide/metformin at reaching the glycemic control goals with less hypoglycemic events in patients with uncontrolled type 2 diabetes mellitus.
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Keywords: Glimepiride; Glibenclamide; Metformin; Combinations; Metabolic control; Glycemic control goals

1. Introduction

Insulin resistance occurs early in type 2 diabetes disease process and may lead to progressive beta cell failure and overt diabetes (Abdul-Ghani, Tripathy, & DeFronzo, 2006). Monotherapy can slow down but does not prevent the progression of the disease. Successful management requires

combination therapy that addresses both insulin resistance and beta cell dysfunction (Cefalu, Waldman, & Ryder, 2007). Clinical trials support the use of combinations of antidiabetic agents with complementary mechanisms of action such as a sulfonylurea/metformin (Dailey, 2003; Rendell, 2004). Early aggressive treatment could improve patient outcomes while reducing overall health care costs.

On one hand, glibenclamide/metformin is the oral antidiabetic combination most used in the clinical practice today (Dailey, 2003); on the other hand, glimepiride—considered as a third-generation sulfonylurea agent—has several beneficial pharmacological effects over glibenclamide, a second-generation sulfonylurea. Glimepiride combined with metformin in a single dose presentation has proved to be effective and safe for type 2 diabetes patients who fail with monotherapy on oral antidiabetic agents (González-Ortiz, Martínez-Abundis, & Grupo para el tratamiento de la diabetes mellitus con combinaciones, 2004).

At present, there is no evidence in medical literature of a comparison between the abovementioned combinations; therefore, the aim of this study was to compare the efficacy of glimepiride/metformin versus glibenclamide/metformin in reaching glycemic control in patients with uncontrolled type 2 diabetes mellitus.

2. Patients and methods

A total of 152 patients with type 2 diabetes mellitus (40 to 65 years of age) were included in a randomized, double-blind, multicenter clinical trial. Patients were overweight or obese [body mass index (BMI) between 25 and 40 kg/m²] and had received monotherapy with metformin (2000 mg/day) and glibenclamide (20 mg/day) or medical-nutritional therapy in accordance with the American Diabetes Association recommendations during at least 3 months. Patients were required to have fasting glucose concentrations of 8.3 to 14.9 mmol/l and hemoglobin A1c (A1C) >7%. Sample size was calculated with a clinical trial formula (Jeyaseelan & Rao, 1989) based on a confidence level of 95%, statistical power of 80%, standard deviation of A1C in uncontrolled type 2 diabetes patients of 1.30%, and an expected difference of 0.65% in A1C. Sample size was 63 patients per group, but it was increased to 76, estimating a 20% loss to follow-up.

Subjects were not included if they presented one of the following: pregnancy, alcohol or drug abuse, insulin treatment, allergy to sulfonylureas or biguanides, heart or renal failure (considered as serum creatinine >133 in males and >124 μmol/l in females), coronary heart disease, stroke, visceral neuropathy, cancer, systemic lupus, erythematosus lupus, or HIV infection. The use of drugs that presented pharmacological interactions with sulfonylureas or metformin was prohibited, with the exception of aspirin (≤100 mg/day).

The following were considered as exclusion criteria, but were statistically analyzed: mild or moderate hypoglycemia (>2 times); severe hypoglycemia (<3.3 mmol/l)

with treatment at minimum doses; hyperglycemia >14.9 mmol/l with treatment at maximum dose during 3 months; severe adverse events; lack of compliance to medical treatment (<80% of prescribed pills were taken); loss of follow-up, >2 consecutive or 3 alternate visits during the study; hospitalization; unauthorized medication; or consent withdrawn.

The following clinical measurements were evaluated: BMI (weight in kilograms divided by height in meters squared), waist–hip ratio, blood pressure, fasting and postprandial glucose (glucose level 2 h after eating a standardized breakfast composed of 72 g of carbohydrates), A1C, high-density lipoprotein (HDL) cholesterol, and triglycerides. If a patient met all selection criteria, randomization was performed to include him or her in one of the following fixed combinations: two glimepiride (1 mg)/metformin (500 mg) pills (Glimetal 1/500, Laboratorios Silanes, Mexico) or two glibenclamide (5 mg)/metformin (500 mg) pills (Bi-Euglucon M5, Laboratorios Roche, Mexico), to be taken with breakfast as pharmacological intervention. The allocation was done by simple randomization by an independent center, and the sequence was concealed until the end of the statistical analyses. The medical nutritional therapy in accordance with the American Diabetes Association was followed throughout the study. If the patient presented an A1C >8% at 3, 6, or 9 months, the dose was doubled (two additional pills with dinner), reaching the maximum dose. In case of obtaining a fasting glucose <5 mmol/l every month, the dose was gradually decreased and the patient is closely observed for hypoglycemia through daily capillary glucose monitoring. Blind coded prescription containers were identical for both drugs. An independent researcher counted the pills in order to evaluate adherence to treatment.

Clinical measurements and fasting glucose levels were evaluated every month, postprandial glucose and A1C concentrations were evaluated every 3 months, and HDL cholesterol and triglyceride concentrations were evaluated every 6 months.

Adverse events were recorded continuously and classified as related or not related to the oral antidiabetic fixed combinations used in each case.

Efficacy criteria included fasting glucose ≤7.2 mmol/l, postprandial glucose <10.0 mmol/l, A1C <7%, or a reduction of A1C ≥1%.

Glucose, HDL cholesterol, and triglycerides were measured using an enzymatic technique (Abbott Laboratories, Abbott Park, IL, USA) with intra- and interassay coefficients of variation <3%. A1C concentrations were estimated using a fluorescent polarization enzymatic method (Abbott Laboratories) with intra- and interassay coefficients of variation <5%.

All subjects signed informed consents in order to participate in the study. The protocol was approved by an ethics committee and met all requirements to perform investigation on human subjects.

3. Statistical analysis

Numerical data were reported as mean and standard deviation, and nominal data were reported as proportion. The analysis was performed by intention to treat. A chi-square test was used in order to compare dichotomous variables. Student's *t* test was used to compare intra- and intergroup differences, and ANOVA test was used to evaluate changes throughout the study. A model of global multiple logistic regression was performed to adjust treatment efficacy (A1C <7 at 12 months) by basal BMI and A1C. *P* values <.05 were considered statistically significant.

4. Results

The glibenclamide/metformin group included 45 women and 31 men, and the glimepiride/metformin group included 48 women and 28 men (*P*=.410). There were no significant age differences between the glibenclamide and glimepiride groups (52.9±7.6 years vs. 52.3±7.6 years, *P*=.618).

BMI changes from the baseline to the end of the study were not significant between glibenclamide and glimepiride groups (0.7±1.5 vs. 0.4±1.7 kg/m², *P*=.254).

There was no significant difference between both groups in the time of evolution of diabetes (4.8±4.8 vs. 4.3±4.7 years, glibenclamide and glimepiride groups, respectively; *P*=.564).

There were no significant differences in clinical and laboratory characteristics between groups at baseline (Table 1).

There was no significant difference between the percentage of patients who increase the dose of drugs throughout the study at any time (for a total of 25% in glibenclamide group and 37% in the glimepiride; *P*=.451).

There were no significant differences between both groups in fasting and postprandial glucose levels throughout the study (data not shown). A significant difference in A1C concentration was observed at the end of the study, where A1C concentration was lower in the glimepiride group (7.6±1.2 vs. 7.2±1.0%, *P*=.025).

No differences were observed between glibenclamide/metformin and glimepiride/metformin groups regarding

	Glibenclamide/ Metformin	Glimepiride/ Metformin	P
Systolic BP (mmHg)	124±12	124±17	.882
Diastolic BP (mmHg)	79±10	76±9	.054
BMI (kg/m²)	29.6±4.3	29.5±4.1	.910
Waist–hip ratio	0.92±0.06	0.92±0.06	.864
Fasting glucose (mg/dl)	11.9±3.1	11.7±4.1	.645
Postprandial glucose (mg/dl)	17.5±4.6	16.8±5.2	.427
A1C (%)	9.6±1.6	9.4±1.8	.473
HDL cholesterol (mg/dl)	1.1±0.2	1.1±0.3	.519
Triglycerides (mg/dl)	2.8±2.3	2.6±1.7	.648

t1.13 BP, blood pressure.

Table 2
Proportion reaching glycemic control goals

	Glibenclamide/ Metformin		Glimepiride/ Metformin	
	6 months	12 months	6 months	12 months
Fasting glucose ≤7.2 mmol/l	45.9	39.4	46.6	46.2
Postprandial glucose <10.0 mmol/l	29.7	18.3	27.4	21.9
Reduction in A1C ≥1%	78.4	76.1	75.3	67.7
A1C <7%	35.1	26.8	50.7	44.6 *

* *P*<.05 between both groups at 12 months.

changes from baseline to the end of the study in fasting (−4.1±3.7 vs. −4.0±4.1 mmol/l, *P*=.945) and postprandial glucose (−4.3±5.8 vs. −4.2±4.6 mmol/l, *P*=.291) and A1C concentration (−2.0±1.5 vs. −2.1±1.6%, *P*=.712).

Table 2 shows the metabolic control goals in both groups at 6 and 12 months; the glimepiride group showed the highest proportion of patients who reached the A1C <7% goal at 12 months of treatment.

The model of global multiple logistic regression to evaluate the efficacy of the treatment (A1C <7% at 12 months), adjusting for basal BMI and A1C, showed that the efficacy persisted in the glimepiride group (OR=2.170, 95% CI=0.034–4.559, *P*=.041), as well as after adjusting for basal A1C (OR=0.346, 95% CI=0.163–0.734, *P*=.006), observing an increased difference between both treatments, as basal A1C increases (A1C <9%: 46.4 vs. 51.7%; A1C ≥9%: 14.0 vs. 38.9%, glibenclamide and glimepiride groups, respectively).

The lipid profile remained without significant changes in both groups throughout the study.

Three patients in the glimepiride group were excluded for the following reasons: car accident, pancreatic cancer, and, in one case, having moved out of the country. Nevertheless, they were included in the statistical analysis. The adherence was 96% and 99% in the glimepiride and glibenclamide groups, respectively. Adverse events were present in 68.4% of patients in the glibenclamide group and in 69.7% of those in the glimepiride group (*P*=.842). The most common adverse events were upper respiratory infectious disease (27.7% in glibenclamide group vs. 28.9% in glimepiride group, *P*=.851) and sensorial abnormalities in lower extremities (28.9% in glibenclamide group vs. 35.5% in glimepiride group, *P*=.318). A higher number (*P*=.047) of mild and moderate hypoglycemic events was observed in the glibenclamide group (28.9%) in comparison to the glimepiride group (17.1%). No severe hypoglycemic events were present in any group.

5. Discussion

It has been clearly demonstrated that when the therapeutic treatment goals for diabetes are not reached, early progression to combination therapy can maintain

228 adequate control of blood glucose in comparison to that
229 achieved with single-agent therapy (U.K. Prospective
230 Diabetes Study Group, 1998). There are very few head-to-
231 head studies that compare the effect between different
232 combinations of antidiabetic agents. In the present study,
233 two fixed metformin combinations were chosen: the first,
234 with glibenclamide, a second-generation sulfonylurea, and
235 the second, with glimepiride, a third-generation sulfonylurea
236 that exhibits effects different from glibenclamide, including
237 several extrapancreatic effects on muscle and adipose cells,
238 elevating active glucose transport and increasing insulin
239 secretion (Müller, Satoh, & Geisen, 1995). There is no
240 information about the comparison of the clinical effect of
241 both sulfonylureas at specific doses; we used two common
242 fixed combinations that could not be equivalent and which
243 could be a limitation of our study. On the other hand, this
244 investigation has methodological strength, obtained through
245 controlling several variables by means of strict selection
246 criteria, as a result of which both groups have similar
247 clinical and laboratory basal characteristics, which did not
248 have significant changes throughout the study. The medical
249 nutritional therapy was permanently evaluated and modified
250 in accordance to the individual characteristics of the
251 patients; however, they did not receive specific indications
252 to increase their physical activity, and this is another
253 limitation of the study, independently of the probability of
254 equilibrium of such characteristic in both groups due to the
255 randomized design.

256 The first clinical experience with the glimepiride/metfor-
257 min combination was published in a study wherein
258 metformin failed as monotherapy; in the same study, a
259 diminution of A1C of 0.7% was reached after 4 months of
260 treatment (Charpentier, Fleury, Kabir, Vaur, & Halimi, 2001).
261 On the other hand, we previously published the use of
262 glimepiride/metformin in a single dose during a 8-month
263 follow-up study, demonstrating its efficacy and safety in
264 patients with type 2 diabetes with secondary failure to
265 glibenclamide. In the abovementioned study, reduction of
266 A1C was of 1.3% and approximately half of the patients
267 showed an A1C reduction of at least 1%. Nevertheless, only a
268 small percentage of patients reached the A1C goal of less than
269 7% (González-Ortiz et al., 2004).

270 In accordance with the new ADA-EASD guidelines, a
271 sulfonylurea combined with metformin constitutes an
272 attractive option in the clinical practice (American Diabetes
273 Association, 2008). This combination can reduce A1C
274 concentration up to 2% (Krentz & Bailey, 2005). Our
275 results showed similar reductions in the fasting and
276 postprandial glucose levels for both antidiabetic combina-
277 tions used in the study, reductions that were evident from
278 the first month of treatment. However, at the end of the
279 study, A1C levels were significantly lower in the
280 glimepiride group than in the glibenclamide group. These
281 findings could be explained by the higher proportion of
282 patients that reached the A1C goal of less than 7% in the

283 glimepiride/metformin group. Besides, it is worth mention-
284 ing that A1C is a better parameter to identify metabolic
285 control, because it evaluates the participation of both basal
286 and postprandial glucose concentrations and their varia-
287 bility (Monnier, Colette, Dunseath, & Owens, 2007).

288 Although we waited for a lower rate of treatment
289 adherence, this was high in both groups and was probably
290 related to the close clinical attention provided by the
291 medical staff.

292 Glibenclamide is well-known to induce a higher
293 frequency of hypoglycemia than other agents, and our
294 results were in accordance with this fact (Rendell, 2004).
295 Other adverse events were considered to be not related to the
296 combination of antidiabetic agents used, probably due to the
297 natural evolution of diabetes.

298 In conclusion, glimepiride/metformin showed a greater
299 efficacy in reaching the metabolic goal of glycemic
300 control with less hypoglycemic events in patients with
301 uncontrolled type 2 diabetes mellitus in comparison with
302 glibenclamide/metformin.

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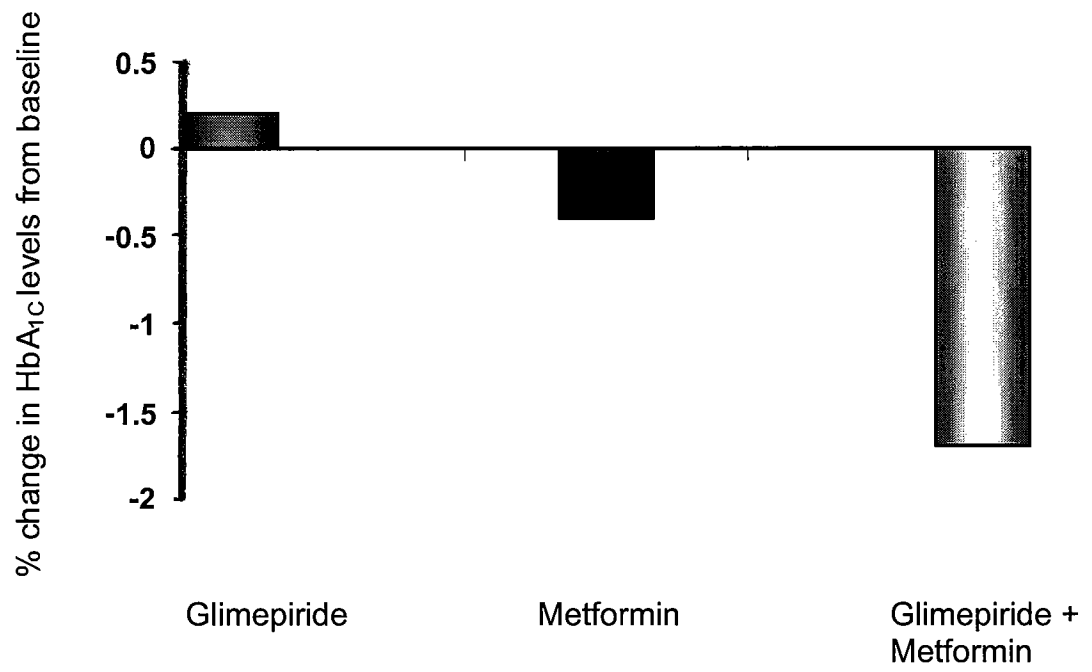
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Exhibit C

Exhibit D

Comparison of effect of metformin in combination with glimepiride and glibenclamide on glycaemic control in patient with type 2 diabetes mellitus

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Abstract

The aim of the present study is to compare the effect of metformin in combination with Glimepiride and Glibenclamide In patient with type 2 Diabetes Mellitus. Subjects and method: This is an open-label, randomized study carried out to study the effect of metformin when it is given in combination with either glimepiride or glibenclamide on glycaemic control in patient with type 2 Diabetes Mellitus. Patients with Glycosylated Hemoglobin more than 7% were included in the study. 31 patients were randomly assigned for treatment based on metformin-glibenclamide 1000/10 mg tablets or metformin-glimepiride1000/2mg for 12 weeks. The comparisons were conducted between these two groups for HbA_{1C}, FPG, PPG and lipid profile. Result: At week 12, the significant reductions in HbA1c were found in both groups but the patients treated with metformin-glimepiride resulted in significantly greater reductions in HbA_{1C} (-1.4%) than metformin-glibenclamide (-1.2%). Also the greater significant reductions were observed in case of FPG, total cholesterole, serum triglyceride and LDL cholesterole in patient with metformin-glimepiride group. Conclusion: Metformin-glimepiride tablets resulted in significantly greater reductions in HbA_{1C} and fasting plasma glucose compared with metformin plus glibenclamide in patients with type 2 diabetes mellitus.

Keywords: combination therapy, glimepiride, glibenclamide, metformin, type 2 diabetes mellitus.

Abbreviation: CVD: Cardiovascular Disease; DM: Diabetes Mellitus; FPG: Fasting Plasma Glucose; Group I: Metformin plus Glimepiride; Group II: Metformin plus Glibenclamide; GIT: Gastrointestinal Tract; HbA_{1C}: Glycosylated Hemoglobin; HDL:

High Density Lipoprotein; LDL:Low Density Lipoprotein;OHAs:Oral Hypoglycaemic Agents; PPG: Post-prandial Plasma Glucose; SEM: Standard Error mean.

Introduction

Diabetes mellitus is a group of metabolic disorders of carbohydrate metabolism in which glucose is underutilized, producing hyperglycemia resulting from a defect in insulin secretion, insulin action, or both.[1,2] It is an endocrine disorder, more than 100 million (6% of the population) of people world-wide are affected inspite of enormous facilities available to control its growth[3]. Type 2 diabetes is caused by two primary metabolic defects: progressive pancreatic β-cell dysfunction and insulin resistance[4]. β-Cell dysfunction superimposed on insulin resistance leads to hyperglycaemia and subsequently to type 2 diabetes. Typically, at the time of diabetes diagnosis, nearly 50% of β-cell function has been lost and less than 60% of normal insulin sensitivity is present[5]. Diabetes is a chronic illness that requires continuing medical care and patient self-management education to prevent acute complications and to reduce the risk of long-term complications[6].The lifestyle modification, diet and exercise of moderate intensity are used to improve insulin sensitivity and are recommended as an integral part of treatment of Type 2 diabetes[7]. When the lifestyle modification, diet and exercise fails to maintain the adequate glycaemic control, oral hypoglycemic agents are introduced as a treatment approach[4,5]. Oral Hypoglycemic Agents (OHAs) can be used either alone or in combination with other OHAs or insulin. The Canadian Diabetes Association 2003 Clinical Practice Guidelines for the Prevention and Management of Diabetes recommends a target hemoglobin A_{1c} concentration of 7.0% or less for all patients with diabetes. Currently, there are five major classes of oral antidiabetic agents: sulphonylureas – insulin secretagogues that target β -cell dysfunction; metformin – a biguanide that reduces hepatic glucose production and improves insulin sensitivity, thiazolidinediones – insulin sensitizers that lower peripheral insulin resistance; α -glucosidase inhibitors – intestinal enzyme inhibitors that slow carbohydrate absorption; and meglitinides – rapid but short-acting, non-sulphonylurea secretagogues[9,10].The goal levels of diabetes related parameters during treatment is given in Table No. 1

Insulin is also important in type 2 DM when blood glucose levels cannot be controlled by diet, weight loss, exercise and oral medications. Insulin is indicated in the following situations: 1) when diet and oral hypoglycaemic drugs fail to control hyperglycaemia and achieve therapy targets 2) diabetes during pregnancy when diet alone is inadequate, 3) when oral hypoglycaemic drugs are contraindicated, 4) during stressful conditions such as infection and surgery[1,2,9].

Table No. 1 Blood-glucose targets for people with Diabetes.

Parameter	Normal	Goal	Action suggested if
Pre-prandial Fasting Glucose	<110 mg/dl	80-120 mg/dl	<80 or >140 mg/dl
2h postprandial Glucose	<140 mg/dl	<140 mg/dl	>180 mg/dl
Bedtime	<120 mg/dl	100-140 mg/dl	<100 or >160 mg/dl
HbA1c	≤ 6%	< 6.5 %	>8 %

Combination therapy: It is always beneficial to switch over the patients on combination therapy, when there is high secondary failure associated with monotherapy and devastating long term consequence of poor glycemic control. A reasonable goal of treatment is to maintain good glycemic control through combination therapy so as to keep HbA1c value below 7% for a particular patient. Initiation of combination drug therapy at low dosages can minimize the side effects associated with high dose therapy of either agent, yield additive clinical benefits, and possibly curtail cost of treatment. For many drugs, 50% of the dosage needed to achieve the maximal therapeutic effect will produce well over 50% of that effect [2, 11].

Patients and method

This was a single center, open-label, randomized parallel group study conducted at the Indira Gandhi Memorial Hospital, Shirpur. (North Maharashtra Region) The study was approved by the local research ethics committee and all subjects gave written informed consent to participate in the study. Patients with age more than 35 yrs, of either sex, glycosylated hemoglobin > 7% and blood sugar level > 140 mg/dl were included in the study. The written consent was also taken from each patient in local language. Patients with current insulin therapy or received insulin for more than six weeks in last 3 months, who had known hypersensitivity to Biguanides and sulphonylurea, who are on chronic medication known to affect glucose metabolism were excluded from the study. Also the patients with renal disease or renal dysfunction, with congestive heart failure, hepatic insufficiency, alcoholic person and pregnant and lactating women were planned to exclude from the study. A total of 31 type 2 diabetic patients were enrolled on a treatment program. The patients were given instructions on diabetic diet and asked to monitor their blood glucose level, both fasting and postprandial, glycosylated hemoglobin and lipid profile at the initial visit to the hospital. The patient's records were maintained for the next three month after their initial visit to hospital. The patients were observed for weight, height and blood pressure measurement. The records of age, sex, family history and other possible

associated diseases were also maintained. The records of the weight and height are helpful for the determination of body mass index. The patients were also interviewed for their initial sign and symptoms. As the patients were recruited for the study they were randomized into two groups according to the treatment they received. Those patients received Metformin-Glimepiride (1000/2 mg/day) combinations were introduced into the group I while those patients received the Metformin-glibenclamide (1000/10 mg/day) were introduced in group II. The patients were asked for the determination of FPG and PPG regularly at the interval of each month. The HbA1C and lipid profile were examined only before the treatment and after 3 months of treatment. The glycosylated hemoglobin determination was carried out by using BIO-RAD Micromat II HbA$_{1C}$ instrument, while FPG, PPG and Lipid profile were determined by using Microplate reader.

Efficacy and safety evaluations

The primary efficacy variable was the change in HbA1C from baseline to 3 month. Secondary efficacy outcomes included changes in fasting plasma glucose, 2-h postprandial plasma glucose and fasting lipid profile levels from baseline to 3 month after randomization of the patient into the study. Safety outcomes included adverse events, particularly hypoglycaemic symptoms. The patients were interviewed and asked for the any type of adverse events throughout the study. The patients were specially asked for the hypoglycaemic symptoms. The daytime hypoglycemic episodes are usually recognized by sweating, nervousness, tremor, and hunger while nighttime hypoglycemia may be without symptoms or manifest as night sweats, unpleasant dreams, or early morning headache.

Statistical analysis

The analysis of Glycosylated hemoglobin and lipid profile was carried out by using the appropriate test. For parametric data, within patient comparisons were made using paired two-tailed t tests and between group comparisons two-tailed unpaired t tests. The FPG and PPG were analyzed by using one way ANOVA followed by Dunnet test.

Results

A total of 31 patients were screened and randomized into the two treatment groups, of whom 28 completed the study successfully. 3 patients, 1 from Metformin-glimepiride group due to withdrawal of consent and 2 patients from Metformin-glibenclamide group were switched to another treatment were excluded from the study. Out of the patients those participated in the study, 48.38% of patients were male while 51.61% of patients were female. A 29.03% of diabetic patients found to be overweight. The patients were categorized as an overweight if their body mass index lies between 25-

9.9 Kg/m². 58.06% of patients were found to be having genetic history of diabetes mellitus. The mean age was 49.95 ± 2.032 yrs. in the first group and was 48.27 ± 2.902 yrs. in the second group. The mean duration of diabetes mellitus was 4.4 ± 0.78 and 4.0 ± 0.57 yrs in Group I and Group II respectively. The baseline characteristics of all patients at randomization are summarized in the table 2.

Table No. 2:- The baseline characteristics of all patients at randomization

	Metformin plus Glimepiride	Metformin plus Glibenclamide
Sex (Male/Female)	10/8	5/8
Age (yrs)	49.95 ± 2.032	48.27 ± 2.902
Diabetes duration (yrs)	4.4 ± 0.78	4.0 ± 0.57
BMI (Kg/m²)	23.13 ± 0.5436	23.85 ± 0.5145
HbA1c (%)	8.9 ± 0.34	9.536 ± 0.41
FPG (mg/dl)	181.8 ± 9.49	205.7 ± 27.48
PPG (mg/dl)	240.0 ± 16.65	301.9 ± 38.62
Total Cholesterol (mg/dl)	150.3 ± 6.82	118.5 ± 6.472
Serum Triglyceride mg/dl)	175.4 ± 17.95	114.0 ± 17.55
HDL (mg/dl)	37.92 ± 1.76	36.43 ± 1.58
LDL (mg/dl)	75.31 ± 6.47	67.21 ± 11.23

Glycaemic control

Glycosylated hemoglobin: - During the study there were no significant differences were found in initial and final values of HbA$_{1c}$ levels of both groups. Though the HbA$_{1c}$ level was found to be reduced more significantly (P: 0.0001) patients treated with Metformin-glimepiride, while in the patients treated with Metformin-glibenclamide also reduced HbA$_{1c}$ but less significantly as compare to Metformin-glimepiride treated patient. The glycosylated hemoglobin was found to be reduced by -1.376 ± 0.27 and -1.227 ± 0.48 in Metformin plus Glimepiride and Metformin plus glibenclamide groups respectively.(Shown in fig No.1)

Fig No. 1:   Comparison of HbA1c between Group I and Group II
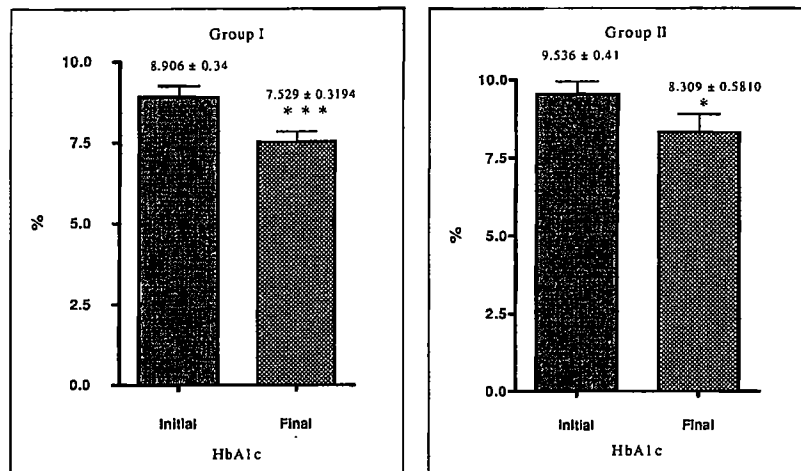
Group I: Metformin plus Glimepiride,Group II: - Metformin plus Glibenclamide.

Data was analyzed by Paired t- test,P<0.001= very significant *** ,

P<0.05 = significant *,Values are expressed as mean ±SEM

FPG AND PPG: - The FPG values were found to be reduced by - 54.59 ± 10.84 mg/dl and - 92.09 ± 24.25 mg/dl in each group I and Group II respectively. The significant reductions in the fasting plasma glucose were found in both groups. The reduction in FPG in the Metformin-glimepiride group was significantly greater than (P: 0.0001) that in the Metformin-glibenclamide group (P: 0.0066) throughout the study. (Shown in Fig No.2)
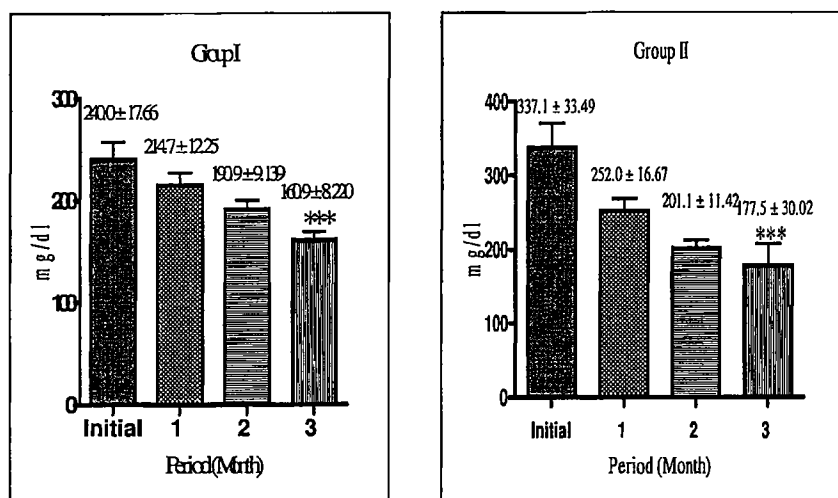
Fig No.2: - Comparison of FPG between Group I and Group.
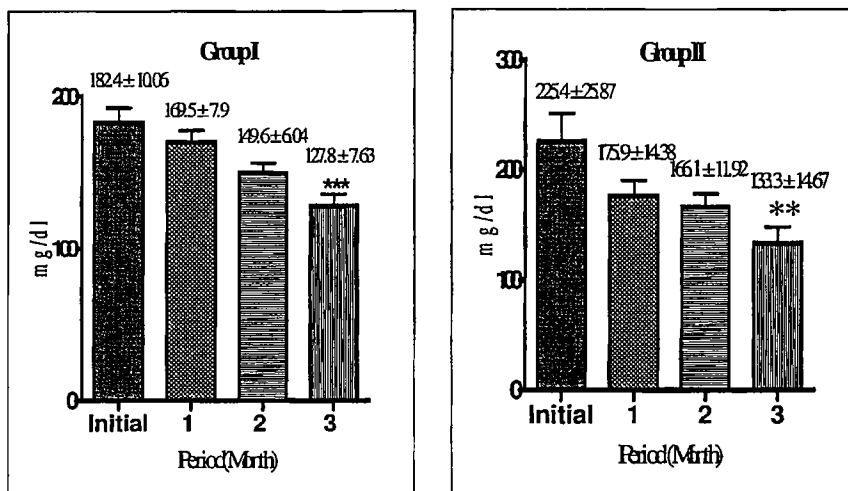
Data was analyzed by one way ANOVA followed by Dunnet test .

$P<0.001$=extremely significant *** ,$P<0.01$= Very significant **,Values are expressed as mean $\pm$SEM

The PPG values were reduced throughout the study period of 3 month by 79.06 mg/dl and 159.64 mg/dl in each group. The PPG values were significantly reduced in both the groups. The significant reduction in PPG in the Metformin-glimepiride group as well metformin glibenclamide group were found similar. ($P<0.0001$) (Shown in fig No.3)

Fig No.3: - Comparison of PPG between Group I and Group II

Data was analyzed by one way ANOVA followed by Dunnet test
$P<0.001$= extremely significant ***
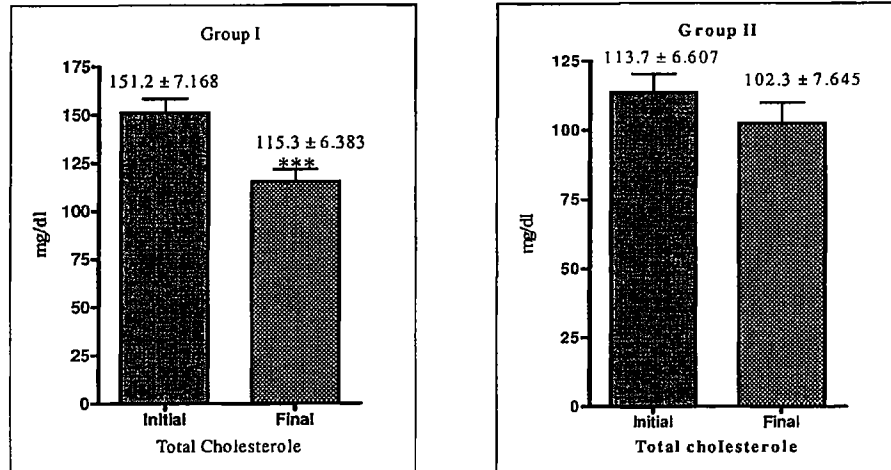Values are expressed as mean $\pm$SEM

Lipid profile

The total lipid profile parameters have been performed in both groups in terms of total cholesterol, serum triglyceride, HDL and LDL.

A significant reduction in the total cholesterol was found in the Metformin and Glimepiride ($P<0.0001$) as compare to Metformin and Glibenclamide group. The total cholesterol was also reduced in the second group but non-significantly. (Shown in Fig No 4)

There were significant reductions (P: 0.0068) found in the Metformin-Glimepiride group in case of serum triglycerides but there were no changes found in the serum
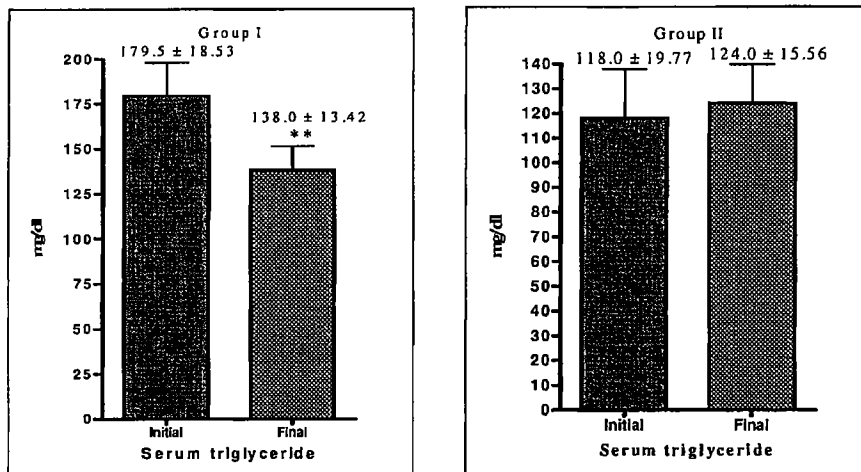
triglyceride level throughout the study in Metformin-Glibenclamide group.(Shown in Fig No 5)

Fig No.4: -Total Cholesterole Group I Vs Group II

Data was analyzed by Paired t test

P<0.001=extremely significant ***

Values are expressed as mean ±SEM

Fig No.5: - Serum triglyceride Group I Vs Group II
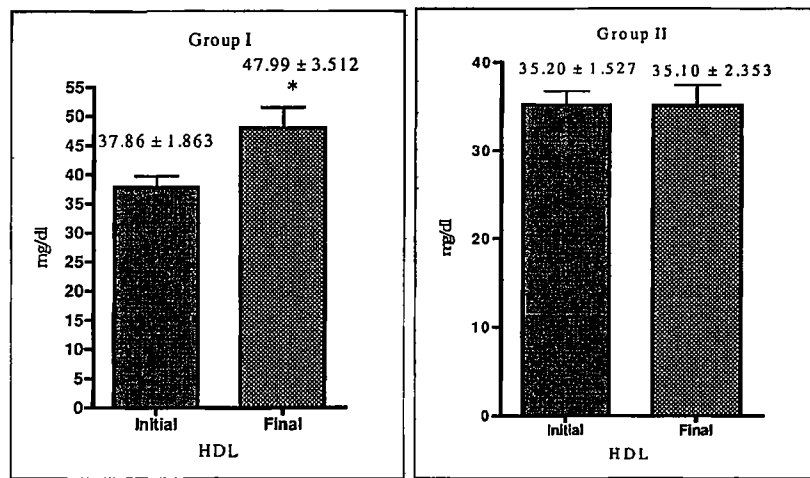

Data was analyzed by Paired t test

P<0.01= very significant ** , Values are expressed as mean ±SEM

The HDL concentration has been found to be increased significantly (P: 0.0190) in the Metformin-Glibenclamide group, however there was no change found in the values of HDL cholesterole in Metformin-glibenclamide group. (Shown in Fig No 6)

The LDL concentration has been found to be reduced significantly (P<0.0001) in the Metformin-glimepiride group. (Shown in Fig No 7)
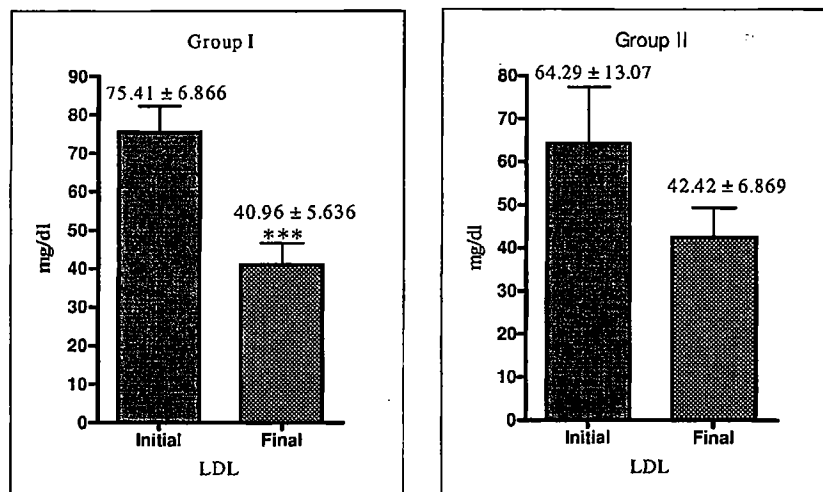
Fig No. 6: - HDL concentration Group I Vs Group II

Data was analyzed by Paired t test
P<0.05 = significant *
Values are expressed as mean ±SEM

Fig No. 7: - LDL concentration Group I Vs Group II

Data was analyzed by Paired t test,P<0.001= extremely significant ***

Hypoglycemic and other adverse effect

The patients were interviewed at the end of the study for the detection of any other side effects. No patient complaint about the any type of nausea, vomiting, headache or GIT side effects at the given doses of medication. However when the patients were asked for the hypoglycemic symptoms, 3 patients from each group reported the hypoglycemic symptoms. It means that 17.64% and 27.27% of patients from Group I and Group II reported the hypoglycemic effect.

Discussion

Type 2 diabetes arises in the settings of insulin resistance in muscle, adipose tissue and liver and a progressive decline in pancreatic β -cell function[5]. A traditional stepwise approach to diabetes therapy involves the use of a single oral agent titrated to maximum dosage, each of which targets a single pathological defect of type 2 diabetes as its primary mechanism of action, with the requirement of poor glycaemic control as an indication for the addition of a second oral agent[13]. The aim of our study was to compare the effect on glycemic control, weight gain, and frequency of hypoglycemia in patients receiving metformin in combination with glimepiride or glibenclamide. During the study it has been found that type 2 diabetes affected both the sex equally and mostly it is pronounced at the age of 49.26 ± 1.604 yrs. while 58% of patients were found to be at the greater risk of hypertension or other cardiovascular complication. Treatment with metformin-glibenclamide tablets and metformin-glimepiride tablets simultaneously targets insulin resistance and insulin deficiency of type 2 diabetes, which may account for the greater effects on glycaemia. Indeed, metformin-glimepiride tablet therapy produced greater mean changes from baseline in HbA1C (1.4% for metformin-glimepiride tablets vs. 1.2% for metformin plus glibenclamide). The greater mean changes from the baseline in case of fasting plasma glucose were found (- 92.09 mg/dl) for metformin-glibenclamide (Group II) while for metformin-glimepiride (Group I) it was (-54.59 mg/dl). But the statistical analysis using one way ANOVA followed by Dunnet test concludes that the Group I reduced FPG is more significantly ($P<0.0001$) than Group II. The metformin glibenclamide combinations were prescribed in patients with fasting plasma glucose more than 240.0 ± 16.65 mg/dl. While the better mean reductions were found in the FPG in patients of group II. Furthermore, 29.41% of patients receiving metformin-glimepiride therapy attained a final HbA1c of <7.0%. These results demonstrate that treatment with metformin-glimepiride was more efficacious than with metformin plus glibenclamide in improving glycaemia by achieving therapeutic goals for HbA_{1C} and fasting plasma glucose in patients with type 2 diabetes. The both combination used in the study are of Biguanide (Metformin) and sulphonylurea (Glimepiride and Glibenclamide) category.

The reductions in the blood sugar level were found in both groups due to the synergistic effect. The synergistic effect of both combinations may be due to the different mechanism of action of individual drugs in the both combination. Metformin decreases hepatic glucose production through inhibition of gluconeogenesis and possibly glycogenolysis and improves the peripheral insulin sensitivity.

In diabetic patients there is an increased risk of cardiovascular complications followed by higher morbidity and mortality than in a nondiabetic population with coronary artery disease. Cardiovascular disease (CVD) is 2–3 times commoner in diabetics than in non-diabetics. Known risk factors are such as raised cholesterol, hypertension, smoking, abdominal obesity, hyperinsulinemia, disorders of platelet function and coagulation, and degree of glycaemic control only partly explain the increased risk[14]. In the present study more than 58% of patients were at the greater risk of cardiovascular diseases. The smoker patients were mostly found to the development of hypertension (66.66%). The patients treated with Metformin-glimepiride combination resulted in the significantly reduction in the total cholesterol, serum triglyceride, and LDL cholesterol while helped to increased the HDL cholesterol throughout the study. So this combination can be considered as the best combination to be prescribed in patients with increased cholesterol and triglyceride concentration.

Conclusion

From the assumption described in results and discussion the present study concludes that the both combinations such as Metformin-glimepiride and Metformin glibenclamide reduced the Glycosylated Hemoglobin level, Fasting and post-prandial plasma glucose significantly. But the metformin-glimepiride combination provided superior control of glycaemia as compare to the Metformin-glibenclamide combination. While the significant reduction in the total cholesterol, serum triglyceride and LDL cholesterol was observed in the Metformin-glimepiride combination. It also significantly increased the HDL cholesterol levels throughout the study period of 12 weeks. So the Metformin-glimepiride combination can be considered as the best combination in patients with increased lipid parameters as compare to metformin-glibenclamide combination in diabetic patients.

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